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CME Review Article

The Respiratory Toxicities of Mustard Gas

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Abstract

Sulfur mustard is one of the major potent chemical warfare agents. It was widely used against not only military personnel but also civilian people of Iran during the last years (1984–1988) of the Iraq–Iran war. A number of studies were performed regarding the acute and long-term consequences of sulfur mustard on respiratory system. Currently, many aspects of leading respiratory disorder that was prescribed as "mustard lung" have been revealed. However, there is growing concern about pathophysiological mechanisms behind the mustard lung. Herein available published materials about mustard lung are summarized, and it has been tried to highlight practical points relevant to the diagnosis and treatment of the disease.

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Keywords ● Sulfur mustard ● chemical weapon ● mustard lung

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Introduction

Sulfur mustard (bis[2-chloroethyl]sulfide; C4H8Cl2S), or as it is commonly called, 'mustard gas', is one of potent DNA alkylating and vesicant chemical warfare agents with the ability to form blisters on the exposed skin.[1] It is a viscous liquid at ambient temperature. It is heavier than water as a liquid, and heavier than air as a vapor. Sulfur mustard was first manufactured in 1822. It was utilized as early as the late 1880s, when it was used as a pesticide and to treat minor tumors. Unfortunately, it was first used as a war gas in 1917, during World War I by the Germans on the British at Ypres. For this reason, sulfur mustard is also called yperite.[2,3] More recent use of sulfur mustard has been by Egypt against Yemen, 1963–1967.[4] There is also some concern that United States military personnel, who served in the Persian Gulf in 1991 during Operation Desert Storm, might have been exposed to sulfur mustard.[5] Soldiers and civil population exposed to a single high dose of sulfur mustard are the main source of studies in this field. Furthermore, in World War I and II occupational studies on Japanese, British and German factory workers provided vast resource about long-term effects of sulfur mustard on workers of sulfur mustard agent manufacturing companies.[6-8]

Sulfur mustard was widely used against both military and civilian people of Iran during the last years (1984–1988) of the Iraq–Iran war.[9,10] Over the time, about 100,000 Iranians were exposed to chemical warfare agents,[9] and more than 34,000 do still suffer from the complications of this warfare, which include ophthalmic, cutaneous and respiratory problems.[11] Among such complications, the respiratory ones have the highest burdens. In the present paper it has been tried to briefly scrutinize various published documents regarding the pulmonary effect of sulfur mustard. Therefore, this review aims at demonstrating the late toxic effects of sulfur mustard on respiratory tract, discussing

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clinical, paraclinical and biomolecular findings of the toxicity, and providing a concise introduction to the available treatments for the affected patients.

From Early Stages after Exposure to Mustard Lung

A number of different studies, including various cross sectional, cohorts and clinical trials have been carried out on the acute and long-term effects of sulfur mustard on respiratory system. There is a large amount of evidence in the human that the respiratory system is one of the primary and main targets of sulfur mustard toxicity following inhalation exposure. The temperature of environment influences the absorption of sulfur mustard. Warm environmental conditions increases the severity of the respiratory adverse effects of sulfur mustard. A series of events following exposure have been studied.[1,3-12] It has been reported that the symptoms of exposure develop over a period of hours to days. Hoarseness and irritation of the nasal mucosa may develop 12 hours to 2 days after the exposure. Recovery may occur after around 2 weeks. Exposure to low dose of sulfur mustard causes sneezing, lacrimation, rhinorrhea and sore throat. Exposure to higher dose may even result in injuries progressing to edema in the pharynx and tracheobronchial tree, followed by death due to severe edema, secondary infection or necrotic bronchopneumonia at early stages. There is evidence that pulmonary injury is the leading cause of mortality in the first few days to weeks after adequately high concentrations of sulfur mustard.[13-15] Earlier, the long-term consequences after exposure to sulfur mustard were categorized in different known disorders such as asthma, chronic bronchiectasis, and pulmonary fibrosis. But the specific nature of related lung pathology remained unknown for years until it was known that bronchiolitis obliterans was the main underlying long-term respiratory consequence after exposure to sulfur mustard.[16,17] Despite the fact that bronchiolitis obliterans in this setting is different from other etiologies, the main pathology is somehow irreversible obstruction of bronchioles. Currently, there is growing interest in the pathophysiological mechanisms behind mustard lung as a unique disorder due to the specific toxic agent.

Pathological Findings

Pathological studies revealed bronchiolitis as delayed respiratory disorders in sulfur mustard-exposed patients.[18-20] In a collaborative histopathological study, surgical (open or thoracoscopic) lung biopsies of patients with chronic respiratory disease resulting from exposure to low or high dose of sulfur mustard were evaluated. In summary, about half of the patients had diagnostic obstructive bronchiolitis or bronchiolectasis and mucus stasis consistent with more proximal luminal compromise. No differences between the low and high-dose groups suggest that the pathological effects of sulfur mustard are not solely dependent on the severity of exposure.[21]

Molecular and Cellular Findings

Molecular and cellular studies are very important in sulfur mustard research field because they provide opportunities to a higher understanding about pathophysiological aspects of the disease, and consequently, will lead to ideal diagnostic biomarkers and novel treatments.

It has been found that the serum levels of IL-8 and IL-6 significantly decreased in the sulfur mustard exposed patients. There was no relation between the serum levels of IL-8 and pulmonary symptoms including chronic cough, dyspnea, sputum and hemoptysis, and pulmonary findings such as crackles and wheezing as well as spirometry parameters. However, there was a relation between the serum levels of IL-6 and wheezing. It was concluded that serum levels of these inflammatory mediators probably could not play a major role in the pathogenesis of pulmonary complications, and it was not related to the degree of severity of pulmonary involvement following exposure to sulfur mustard.[22]

Sulfur mustard toxicity and pathogenesis appears to be mediated by the generation of reactive oxygen species as well.[23-25] The alpha$_1$-antitrypsin activities in patients with respiratory disease following exposure to sulfur mustard were lower than control groups, but there was no phenotypic alterations in this group of patients. It was concluded that the difference in the clinical pulmonary symptoms of the two groups was attributed to diminished alpha$_1$-antitrypsin activity due to oxidative stress.[26] Moreover, superoxide dismutase activity in the healthy control group was higher than that in the moderate-to-severe group of sulfur mustard exposed patients. The catalase activity in the healthy control group was lower than that in the moderate-to-severe group or in the mild group.[27] It reflects oxidant-antioxidant

imbalance as one of the pathological mechanisms underlying lung injuries.

One of the suggested mechanisms of sulfur mustard-induced cytotoxicity is alkylation of glutathione (GSH). Alkylation removes one of the major cellular defense mechanisms against electrophilic compounds and oxidants. While GSH is depleted, a series of events lead to cell damage and death. On other hand, the increase of cellular GSH levels decreases the toxic effects of sulfur mustard in human peripheral blood lymphocytes.[23] It was proposed that the modulation of GSH levels within the cell may help to reduce the cytotoxicity of sulfur mustard when used as pretreatment.[28]

Malondialdehyde (MDA), which arises from the breakdown of lipid peroxyl radicals, is one of the indicators of oxidative stress. It can cause further oxidative injury by oxidizing protein molecules, thus it is both an indicator and effector of oxidative stress. Sulfur mustard injection caused oxidative stress, as reflected by dramatically increased levels of the lipid peroxidation end product.[29] Increased MDA levels depict increased lipid-peroxidation, which may be due to the excessive production of free radicals after exposed to sulfur mustard.[30] It was concluded that the determination of serum MDA level may be helpful for the diagnosis and treatment of pneumonia.[31] According to addressed evidences, Shohrati and colleagues designed a study to measure the level of GSH and MDA activities in patients intoxicated with sulfur mustard and to evaluate the relationship between their activities and the severity of pulmonary dysfunction. They found such an imbalance in the oxidative-antioxidative system in patients suffering from sulfur mustard-induced lung injuries, as shown by decreased serum level of GSH and increased level of MDA. Individuals with moderate-to-severe sulfur mustard-induced lung injuries showed a higher tendency for the decreased level of GSH and increased level of MDA than those with mild injuries. However, there was only minimal association between the pulmonary function parameters and serum level of MDA and GSH.[32]

Analysis of expressed proteomic proteins patterns in bronchoalveolar lavage (BAL) fluid of sulfur mustard-exposed patients was compared to a group of control healthy patients.[33] There was a significant increase in vitamin D binding protein isoforms, fibrinogen, and haptoglobin isoforms especially in patients with moderate and severe lung diseases. Furthermore, calcyphosine, surfactant protein A (SPA) and transthyretin were decreased in these patients. Calcium-binding proteins were reported as the main proteins involved in the process of sulfur mustard pathogenecity.[33] The isoforms of S100 protein are implicated in the immune response, differentiation, cytoskeleton dynamics, enzyme activity, Ca^{2+} homeostasis and growth.[34] Also neutrophils were significantly increased in patients that were severely affected. It was concluded that it might be due to an increase in S100 A8 level or vice versa. This protein may have a protective role against lung tissue damage. Furthermore, it could help differentiate the severity of the damage in exposed patients. Calcyphosine, another calcium-binding protein, is involved in cell growth and differentiation, and may regulate essential cell functions like proliferation and differentiation as well as cell degranulation.[35] A significant reduction in surfactant-associated protein A (SPA) isoforms, which was correlated with the severity of pulmonary dysfunction, was noticed in mustard intoxicated patients. It was assumed that not only sulfur mustard directly damaged the lung tissues, but also it might play a destructive role on the lung protective factors. Apolipoprotein A1 was detected in all patients' BAL fluid, but none of the healthy controls. A significant increase in Apo A1 and haptoglobin isoforms was observed. The increase in these proteins was associated with the severity of pulmonary dysfunction. Furthermore, S100 calcium-binding protein A8 was only detected in BAL fluid of moderate and severe groups. Interestingly, even mild group with low damage also showed an increase in Apo A1 expression as an indicator of pulmonary dysfunction. In contrast, S100 calcium-binding protein expressed dominantly in moderate and severe groups, but not in the mild group. Several studies have suggested an antioxidant effect for these two proteins.[36,37] Finally, an increase in S100 A8 and a decrease in calcyphosine calcium-binding proteins were suggested as a biomarkers for sulfur mustard induced lung damage.[33]

The Renin Angiotensin System has been implicated in lung inflammatory and fibrotic responses. Genetic variation within the gene coding for the Angiotensin Converting Enzyme (ACE), specifically the Insertion/Deletion polymorphism (I/D), is associated with variable levels of ACE and with the severity of several acute and chronic respiratory diseases. In a study on 208 Kurdish patients who had suffered high exposure to mustard gas in Sardasht, Iran, ACE Insertion/Deletion genotyping was performed. ACE genotype was determined in 207 subjects. The ACE D allele was associated with higher forced expiratory volume in 1 s

(FEV1) % when assessed 18 years after high exposure to mustard gas.[38]

Clinical Manifestations

Early respiratory effects of sulfur mustard exposure were mentioned earlier. In long-term wheezing, rale, crepitation and decreased respiratory sounds are the major clinical signs in these patients.[39,40] The chronic cough is the most common complaint in these patients. Similar to chemical-induced chronic bronchitis,[39] bronchospasm, postnasal drip syndrome, and gastroesophageal reflux disease are known as the three main causes of chronic cough in the patients with chronic bronchitis.[41]

Pulmonary Function Tests

Pulmonary function test (PFT) is normal in the majority of patients with low-dose exposure, who developed respiratory complications in the long-term. It was followed by mild obstructive involvement.[42] In a study of 34,000 of people poisoned by sulfur mustard, it was revealed that more than half (57.5%) of the exposed patients developed normal PFT, based on American Thoracic Society Criteria.[11] Of patients suffering from respiratory problems, 37% had mild, 4.5% had moderate, and 1% had severe pulmonary function impairment.

The obstructive pattern is the most common feature in patients who show pulmonary involvement. Somewhat less prevalent patterns are occasionally mixed restrictive and obstructive, and purely restrictive patterns.[40,43,44] One decade of follow-up in sulfur mustard-exposed patients revealed that the lung capacity changes in the FEV1 decreased about 50 ml/year.[45]

Imaging Findings

Most of the symptomatic patients have normal or nonspecific changes in the chest x-rays.[46,47] Suggestive findings of bronchiectasis like increased bronchovascular and interstitial markings, bronchial wall thickening, and hyperaeration are neither specific nor diagnostic. While the chest high resolution computed tomography (HRCT) provides more specific characteristics for the diagnosis of mustard lung, air trapping and mosaic pattern are the most frequent radiological findings in both symptomatic and asymptomatic patients.[19,21] Bronchiectasis and increased thickening of bronchial wall can be detected in chest HRCT images.[19,47] In a retrospective cohort study, the severe and moderate groups had a similar frequency of obstructive pattern (21%), whereas only one patient in the mild group showed this pattern. Air trapping did not significantly differ between the groups. In the mild group, 74.8% showed significant air trapping, whereas it was 62.3% in moderate and 67.0% in severe groups. In fact, moderate and severe exposure to sulfur mustard causes equal risk of late pulmonary complications, while mild exposure has lesser risk.[48]

Carcinogenicity

No finding had been detected to support the malignancy in patients with protracted hemoptysis in association with history of single exposure to sulfur mustard using imaging and pathological evaluations. Cytological investigation of bronchial lavage for malignancy in all cases was negative. It has been concluded that hemoptysis per se in acutely exposed sulfur mustard patients is not a valuable evidence of lung malignancy. Anyhow, a close monitoring of these patients for early detection of any kind of malignancy is still recommended.[49]

In a historical cohort study 500 male veterans with single high dose exposure during Iraq-Iran war were compared to veterans without exposure with same demographic characteristics. Only 3 cases of cancer, two lung cancer and one lymphoma, were detected in the exposed group. The relative risk of cancer was 4.02 (95% CI=0.45–36.1), and there was no statistically significant difference between incidence of cancer in exposed and non-exposed groups.[50] In another Cohort study on 500 individuals including 372 civilian populations from Sardasht-Iran no case with lung cancer has been reported.[51]

The p53 immunoreactivity was evaluated as a diagnostic marker in bronchial epithelium of individuals with histories of tobacco use and/or sulfur mustard exposure to define late pulmonary complications of sulfur mustard. Initial data trends suggest an additive contribution of sulfur mustard exposure and smoking to p53 immunoreactivity. Among nonsmoking participants, those with sulfur mustard exposure exhibited lower p53 immunoreactivity than did those without previous sulfur mustard contact.[52]

It is well documented that prolonged exposure to sulfur mustard even at low dose is associated with an increased risk of lung or other respiratory tract cancers.[53-62] However, there is no such a strong and sufficient evidence for acute single high dose exposure. The fact that whether or not the small number of reported lung cancer after single high dose exposure are due to direct induction of malignancy of

sulfur mustard or are caused by confounding factors such as smoking, which is considered a definite carcinogen, could not be concluded.[63]

Treatment

Conventional therapies containing corticosteroids, antibiotics, mucolytics, long-term oxygen therapy and physiotherapy are prescribed for this setting. However, such different medications have no desirable effect and also have known side effects.[16] Although no cure has been yet been found for the mustard lung and the drugs have side effects, these different treatments are used to decrease symptoms.

Bronchodilators are effective in improving the lung function, especially in cases with moderate to severe pulmonary obstruction.[64] With a combination of beta-agonist agents, such as Sulbotamol, and an anticholinergic, such as Ipratropium bromide, a greater bronchodilation can be achieved compared with administration of these drugs alone.[65] In a phase III prospective randomized clinical trial, inhaled corticosteroids and long-acting beta 2-agonists were effective in the treatment of patients with chronic bronchiolitis following exposure to sulfur mustard. However, a medium dose of fluticasone/salmeterol, rather than a very high dose of beclomethasone with only the short-acting beta-agonist, had the same effect on the airways reversibility.[66]

Because of anti-inflammatory and antioxidants effects of macrolides, beside their antibacterial activity, these drugs have been used for the treatment of this group of patients.[67] In an open-labeled clinical study, clarithromycin and N-acetylcysteine were administrated concomitantly for 6 months in sulfur mustard-exposed patients with chronic bronchitis and bronchiolitis obliterans who were nonresponsive to conventional treatments. Subsequently, significant improvement was observed in coughing and sputum production in all patients. Also, the FEV1 and forced vital capacity were noticeably improved.[68]

The oral corticosteroid therapies are recommended for occasions of the disease exacerbation. Moreover, because of adverse effects of long-term corticosteroid therapy, we restrict its administration to those who have received beneficial effects of short-course therapy in exacerbation occasions.[66]

N-Acetylcysteine is a potent antioxidant and mucolytic agent that acts as a pro-drug for cysteine and glutathione. In a double-blind clinical trial, dyspnea, wake-up dyspnea, and cough improved after 4 months of N-acetylcysteine administration compared to the placebo group. N-acetylcysteine reduced sputum and improved Spirometric components significantly in N-acetylcysteine group compared to the placebo group.[69]

In a randomized triple-blind controlled cross-over clinical trial the efficacy of inhaled furosemide (4 ml equal to 40 mg in 10 min) with placebo (4 ml of 0.9% saline solution) was compared in 41 mustard gas-exposed patients. The findings, however, failed to address the previously reported effects of inhaled furosemide on dyspnea.[70]

It has been reported that interferon gamma can be used in nonresponsive cases. An improvement of PFT and dyspnea indices, a decrease in hospitalization time, and an increase in arterial oxygenation could be achieved with interferon gamma 1-b administration.[71]

Furthermore, due to the irreversible nature of their disease these patients do not respond well to bronchodilators. Consequently, it is reasonable to look for new drugs and protocols in order to substitute the old ones. While currently inhaled corticosteroids and bronchodilators are cardinal treatments in chronic obstructive pulmonary diseases, they haven't had desirable effects, and have just been able to control the symptoms without cure. Because of the irreversibility of bronchiole obstruction the use of some modalities like noninvasive ventilation has some benefit.[72]

Conclusion

The present paper reviewed selected practical resources on both pathophysiological and clinical aspects of mustard lung. There are enormous questions that are left without answer, and more researches are required to answer those questions. Further investigations are necessary to reveal exact nature of the disease and to find new biomarkers. Identification of the genes that are responsible to pathogenesis, and the modulation of such genes are the main aims for diagnosis and treatment of the disease.

Conflict of Interest: None declared

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